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Green plants that feed on fungi

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Green plants that feed on fungi: facts and questions about mixotrophy

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Several green, photosynthetic plants in orchids and Ericaceae were recently found to recover carbon from the mycorrhizal fungi associated with their roots, a dual nutritional capability called mixotrophy. The physiological and cellular processes allowing carbon gain from the fungus are not well understood. We believe that this phenomenon is overlooked and propose several land plant families and ecosystems that should be investigated for possible mixotrophy. We speculate that mixotrophy allowed, in some lineages, the evolution of heterotrophic plants, that is, non-photosynthetic plants that obtain their carbon from organic compounds. Moreover, the amount of carbon gained from the fungus varies from one site to another in mixotrophs. Drawing a parallel with mixotrophy in planktonic algae, we propose some hypotheses that could account for this.

A newly discovered form of plant nutrition

A recent breakthrough in the understanding of plant nutrition is the discovery that some green plants from temperate forests not only perform photosynthesis but also obtain additional carbon (C) from their symbiotic fungi. This second C source results from the exploitation of existing mycorrhizal symbioses that link soil fungi with the roots of *circa* 90% of land plants [1]. In the typical mycorrhiza, the fungus provides mineral resources, which are collected by the fungal soil mycelium, and receives plant photosynthates as a reward. However, some green orchids (in *Epipactis*, *Cephalanthera*, *Plantanthera* and other genera [2–5]) and, more recently, some small green perennial shrubs from the Ericaceae family (in the genera *Pyrola*, *Orthilia* and *Chimaphila*, collectively referred to as pyroloids [5,6]) have been shown to receive variable amounts of C from their mycorrhizal fungi.

Plants using fungal C are not new to science: several non-green plants are already known to rely solely on C from their mycorrhizal fungi (Box 1) [7]. These plants are called mycoheterotrophic because mycorrhizal fungi support their heterotrophy. Indeed, mixotrophic orchids and pyroloids are phylogenetically related to some mycoheterotrophic species, as discussed below. The true breakthrough in plant nutrition reported here is the finding of a dual nutrition in plants, partly auto- and partly heterotrophic. Here, we summarize how two investigation tools (stable isotopes and molecular identification of the fungi) have provided new insights into this phenomenon.

Furthermore, we suggest that these tools now allow the question of the general relevance and ecological importance of mixotrophy to be addressed. We propose some research hypotheses on its ecological meaning and evolution.

The diversity of mixotrophic eukaryotes

C availability is a limiting factor for many organisms. In autotrophs, light availability might also limit C nutrition, and many photosynthetic lineages obtain additional C in a heterotrophic manner. The resulting mixotrophy was long ago reported and studied for planktonic algae that show a wide range of variation, from autotrophs with facultative heterotrophy to heterotrophs that are facultatively autotrophic [8]. In some algae, C is obtained from dissolved organic matter: *Chlamydomonas reinhardtii* grown on acetate derives 50% of its C from this source [9]. Most often, mixotrophy in algae is achieved by phagocytosis of small planktonic prey, a strategy found in various, phylogenetically unrelated eukaryotic taxa, such as Chlorarachniophyta, Dinophyta, Ciliates, Haptophyta and Cryptophyta [10]. Planktonic algae achieve up to 95% of the bacterivory in the superficial ocean layer [11]. In these algal lineages, phagocytosis is a conserved ancestral trait that previously allowed nutrition of heterotrophic ancestors and, at a certain time, allowed the engulfment of free-living autotrophic cells that evolved into plastids [10].

Glossary

Autotroph: an organism that is able to use atmospheric CO_2 as its sole carbon source, for example by way of photosynthesis.

Hemiparasite: a plant, such as mistletoe, that although it is capable of performing photosynthesis, lives parasitically on other plants, from which it obtains mineral nutrients and water. In some cases, mixotrophy occurs: the hemiparasites obtain carbon compounds from the sap of the host.

Mixotroph: an autotrophic organism that combines its photosynthesis and a partial heterotrophy as carbon sources (synonyms: hemi-autotroph or partial mycoheterotroph). Indeed, a continuum from autotrophic to fully heterotrophic organisms exists in nature.

Mycoheterotroph: a non-photosynthetic, non-chlorophyllous plant that obtains not only minerals but also carbon from its mycorrhizal fungus. Mycoheterotrophs were previously called 'saprophytic plants' or 'saprobic plants' (see Box 1).

Mycorrhiza: a symbiotic association between a soil fungus and a plant root. It is usually a mutualism in which plant photosynthates are exchanged for mineral resources acquired by the fungus from the soil, but some plants, such as mixo- and mycoheterotrophs, can reverse the carbon flow.

Phagocytosis: a cellular process by which structures are engulfed and digested in a eukaryotic cell, for example hyphae penetrating host cells in some orchid mycorrhizae, or prey acquired by unicellular predators.

Pyroloids: members of a tribe (*Pyroleae*) in the family Ericaceae, species of which have been recently shown to be mixotrophic. A sister clade to the two mycoheterotrophic Ericaceae tribes *Monotropeae* and *Pterosporeae*.

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Box 1. Mycoheterotrophy, a heterotrophic lifestyle that has frequently arisen in plant evolution

More than 400 heterotrophic plants receive all their nutrients, including carbon (C), from their mycorrhizal fungi [7]. These plants were once considered as saprobic, but due to the fungal origin of their nutrients, they are now called mycoheterotrophs [7,46]. Much recent work has focused on the identification of their mycorrhizal fungi. In more than 30 studied species, two common features were observed: each mycoheterotrophic plant is associated specifically with a fungal clade (a genus or a sub-group within a genus) and the fungi involved are also mycorrhizal on surrounding autotrophic plants (a few tropical orchids might, however, associate with soil saprobic fungi [47]). This high specificity between symbionts contrasts with the low specificity in mycorrhizal associations of non-mycoheterotrophic plants, where a fungal species is associated with several plant species and vice versa [1]. Mixotrophic plants show either specific [21,22] or non-specific [3–6,25,29] mycorrhizal associations. Reasons for mycoheterotrophic specificity remain debated, but it is often considered that an evolutionary arms race in a parasitic context can lead to specialization [46], when a mycoheterotrophic plant evolves to parasitize the fungus and the fungus reciprocally evolves to get rid of it. Unfortunately, so far we have no direct evidence that mycoheterotrophs are detrimental to their mycorrhizal fungi, and parasitism is thus not demonstrated.

For most mycoheterotrophic plants, the fungus providing C is also mycorrhizal on nearby autotrophic plants; indeed, labelling experiments demonstrated that these plants are the ultimate C source [48], by way of hyphal links. Thus, a mycoheterotrophic plant is equivalent to a mixotrophic plant that would not rely on photosynthesis for C supply, but only on fungal C (Figure 1b in main text). As a result, ^{13}C abundance in the mycoheterotrophic plant and the associated mycorrhizal fungi is the same (see *Monotropa hypopitys* in Figure 1a in main text). Although absolute values of ^{13}C abundance vary from one site to another, the observed difference between mycoheterotrophic and autotrophic plants from the same site is relatively stable ($\sim$7‰ in δ^{13}C [21]).

All available data indicate that shifts from mixotrophy to mycoheterotrophy are likely to have occurred in evolution. With increasing knowledge of the phylogenetic position of mycoheterotrophic plants, there is some evidence that they arose repeatedly from mixotrophic ancestors (Figure I). So far, this has been documented in orchids [3,4,25] and pyroloids [5], where mixotrophy has been demonstrated. In the series of states leading from autotrophy to mixotrophy and then to mycoheterotrophy, reversal from mixotrophy to full autotrophy is suspected to occur ([29], see *E. palustris* in Figure I). By contrast, reversal from mycoheterotrophy to mixotrophy has not been documented, perhaps because of the irreversible alteration of photosynthetic genes in mycoheterotrophs [49].

Figure I. A phylogeny of the orchid tribe Neottieae supports a series of states leading from autotrophy to mixotrophy and then to mycoheterotrophy (tree based on *rbcL*, ITS and *trnS-G* by maximum likelihood method; red asterisks indicate branches supported by >85% bootstrap values after 1000 replicates). The most parsimonious ancestral states are represented; the following colour code is used for names and branches: black, unknown trophic state; orange, mycoheterotrophic; blue, mixotrophic; green, autotrophic.

By contrast, mixotrophy among land plants is poorly studied and seems to be a secondarily, recently derived feature [5] (Box 1). It was sometimes reported as 'hemiautotrophy' or 'partial heterotrophy', but the broader term 'mixotrophy' unifies the diverse occurrences of organic C acquisition by photosynthetic eukaryotes in all ecosystems. Moreover, unifying all mixotrophic models allows the formulation of hypotheses for land plants starting from the better known algal mixotrophy.

Mixotrophy has long been known in hemiparasites, that is, plants that parasitize other plants but retain photosynthetic abilities. Besides producing their own photosynthates, some hemiparasites gain organic C from host plants [12]. For example, mistletoes derive up to 63% of their C from their host [13]. The possibility that some carnivorous plants derive C from their prey [14], as a side-product of the absorption of organic nitrogen (N) and phosphorus (P), has also been reported, but somewhat overlooked [15]. However, parasitic and carnivorous plants have evolved specialized structures (such as haustoria or
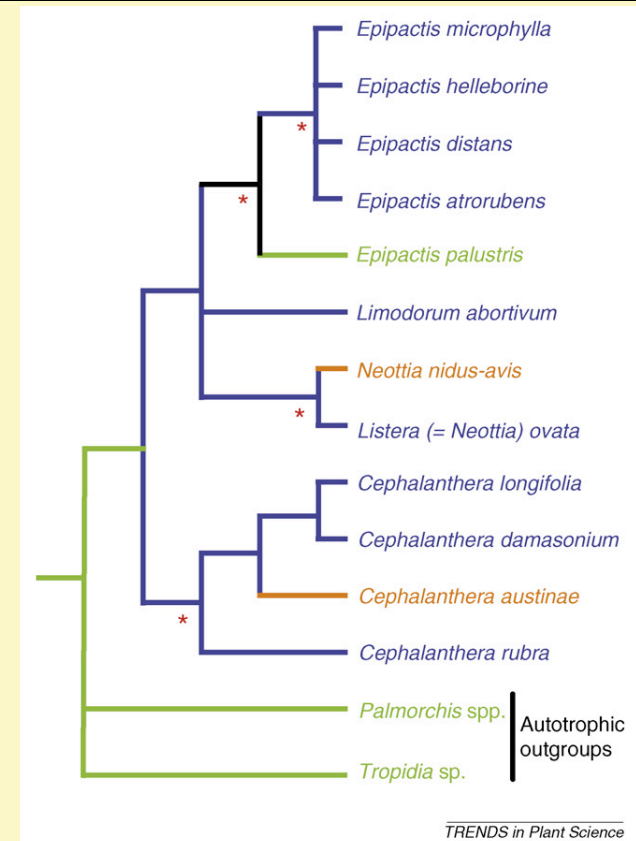
trapping devices) and occur only in a few families, whereas the newly discovered mixotrophy in orchids and pyroloids simply relies on mycorrhizae, a symbiosis already present in most land plants [1]. It is thus potentially a more widespread strategy. For simplification, this mycorrhizal mixotrophy is called mixotrophy throughout this text.

Plant carbon supply from two distinct sources
^{14}C or ^{13}C labelling experiments have demonstrated that various photosynthetic plants can receive C from their fungal associates [1,16,17]. However, such methods only report instantaneous transfers, which might not occur continuously or under all conditions [18,19]. Moreover, they do not account for the exact contribution of such transfers to the plant's C budget over the whole growing season. By contrast, natural abundance in ^{13}C in plant biomass is a powerful tool for unravelling food sources *in situ* [14,20]; food sources over the whole lifespan of the plant can be interpreted without experimental disturbance. Compared to neighbouring autotrophic
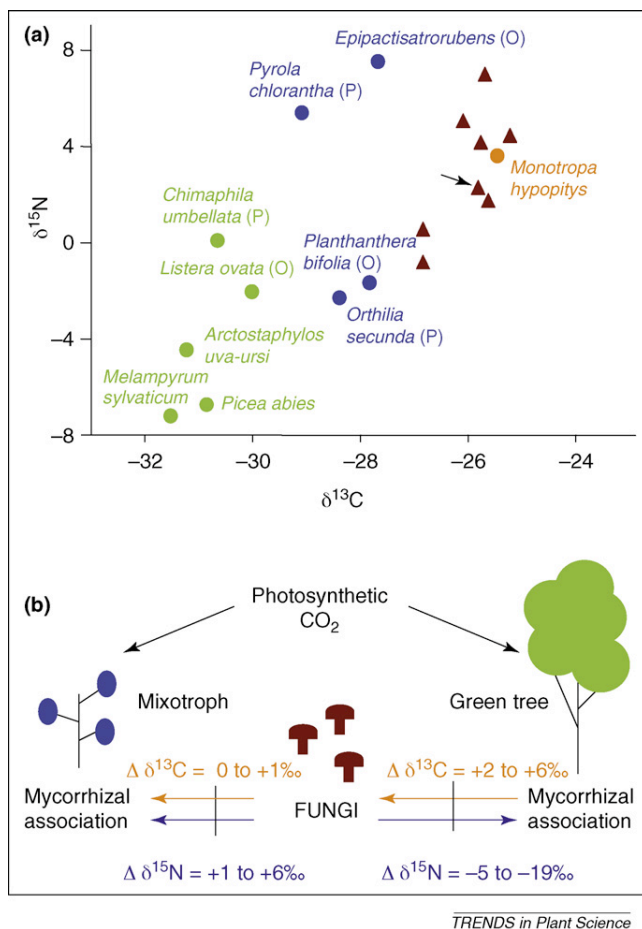
Figure 1. Stable isotope abundance in mixotrophic and mycoheterotrophic plants. Isotope abundance is expressed in $\delta^{13}C$ and $\delta^{15}N$ values (in parts per thousand) relative to international standards: $\delta^{13}C$ or $\delta^{15}N = [(R(sample)/R(standard) - 1] \times 1000$, where R is the molar ratio (i.e. $^{13}C:^{12}C$ or $^{15}N:^{14}N$) (reproduced from Ref. [5], with permission). **(a)** Stable isotope abundance in plants and mycorrhizal fungi from a boreal Estonian forest (reproduced from Ref. [5], with permission). Species include mixotrophic and autotrophic orchids (O), as well as pyroloids (P) and Ericaeae phylogenetically related to pyroloids (the autotrophic *Arctostaphylos uva-ursi* and the mycoheterotrophic *Monotropa hypopitys*). Code for colours: brown, mycorrhizal fungi associated with surrounding trees and mixotrophs; orange, mycoheterotrophic plants; blue: mixotrophic plants; green: autotrophic plants. The fungus indicated by an arrow (*Tricholoma myomyces*) is mycorrhizal on *M. hypopitys*. **(b)** A summary of C (orange) and N (blue) nutrient flow in mixotrophic plants and associated plants (green tree) and fungi, with ranges of isotopic fractionation at the various plant–fungus interfaces (see Ref. [6] for a review).

plants, mixotrophic orchids and pyroloids are enriched in ^{13}C, although they perform the same C3 photosynthesis (Figure 1a) [2–6,21]. The high amount of ^{13}C in mixotrophic plants tends to be close to the amount of ^{13}C found in their mycorrhizal fungi, a situation reported for mycoheterotrophic plants (Box 1). ^{15}N abundances are also congruent with the use of fungal resources (Figure 1a). This isotope becomes more concentrated along most trophic chains: fungi that often exploit substrates derived from plant substrates (directly or after recycling) are richer in ^{15}N than autotrophic plants. In turn, plants feeding on fungi are richer in ^{15}N than their mycorrhizal fungi [2]. Due to their mixed nutrition, mixotrophic plants have ^{15}N abundances that lie above those measured in autotrophic plants and close to those measured in mycorrhizal fungi (Figure 1a).

Whenever gas exchanges have been investigated, mixotrophic plants have demonstrated the ability to fix atmospheric CO_2 through photosynthesis (Figure 1b, 2) [4,5,22]. However, depending on the species, low light conditions [4], low chlorophyll content [21] or low photosynthetic activity [22] limit the plants' photosynthesis rate to a level equal to or lower than their respiration. These plants thus clearly need an additional C source for growth and reproduction. In some orchids, for example *Corallorhiza trifida* [21] and *Limodorum abortivum* [22], strong reduction of leaf size and number also limits photosynthesis. As a result, a large range of variation in the level of heterotrophy (the plant's dependence on the fungal C) can be expected.

This was further measured by ^{13}C abundances in mixotrophs, whose biomass results from mixing a proportion (*p*) of fungal C with (1-*p*) of photosynthetic C. Using the ^{13}C abundances in mycoheterotrophic and autotrophic plants as references, *p* was calculated to range from 0 to 85% in investigated mixotrophs [2,5,6]. As expected, this value varies among species as well as from one site to another for a given species [2,5,6]. A major limitation of these calculations is that they establish the contribution of fungal C to biomass (anabolism) but not to catabolism. Investigations on respiratory CO_2 will thus be necessary to build a global view of C metabolism in mixotrophs.

The fungal symbionts: carbon donors and recipients
Molecular methods are now widespread in microbial ecology and are especially useful in identifying poorly cultivable mycorrhizal fungi. Mycologists have developed barcoding methods based on fungal rDNA, for which reference sequences exist in public databases [23]. Identification of the mycorrhizal fungi of mixotrophs is more than a purely descriptive task because the putative ecology of these fungi provides clues to the ultimate C source that is being exploited. The mycorrhizal fungi that are associated with pyroloids and orchids belong to diverse fungal taxa (Ascomycetes and Basidiomycetes) that usually form mycorrhizae on tree roots [3,5,6,22,24]. In some reports, they were simultaneously detected on surrounding trees of the study site [4,25]. Mixotrophic plants that live in forests thus derive photosynthates from overstory trees to supplement their photosynthesis, by way of sharing fungi mutually (Figure 1b). This adds to the growing body of evidence that coexisting plants share mycorrhizal fungi that mediate plant–plant interactions [17,18], so that some plants can evolve adaptations to use the resulting plant–plant links.

It is noteworthy that, so far, no published labelling experiment has directly supported the mixotrophic scenario of Figure 1b, although such experiments have already demonstrated C transfer from surrounding trees to mycoheterotrophic plants [18]. Moreover, mechanisms transferring fungal C to mixotrophs (and mycoheterotrophs) remain fully unknown. In orchid mycorrhizae, fungal hyphae colonize the root cells and finally undergo lysis [1] of unclear role: it can be interpreted as mobilization of nutrients or as a simple recycling of old structures. An appealing scenario considers it as a phagocytosis-like process mobilizing fungal C. This well explains why the ^{13}C
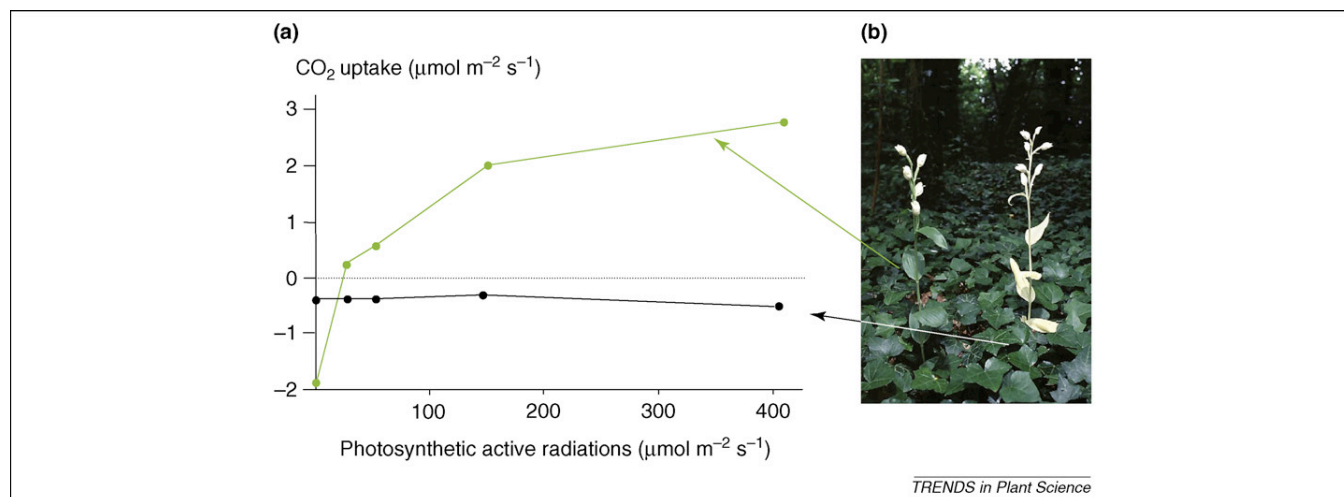
Figure 2. CO_2 uptake in response to light levels **(a)** in *Cephalanthera damasonium*, a mixotrophic, green orchid species in which non-chlorophyllous variants exist that survive as mycoheterotrophs. **(b)** A typical green *C. damasonium* individual is pictured together with a non-chlorophyllous variant (on the right), which shows no CO_2 uptake due to the lack of any photosynthetic ability. Reproduced from Ref. [4], with permission.

content of the receiving plant is similar to that of the associated fungi, because hyphae are totally digested and absorbed [26]. Two facts contradict the digestion model, however: ^{15}N values differ from associated fungi in mycoheterotrophs [26] and, although fungal hyphae also penetrate root cells of pyroloids, no lysis of fungi has been observed in these species [5,24]. Thus, other transfer mechanisms might act in the mobilization of fungal C. An alternative scenario involves transfer of organic molecules from living hyphae to host cells. An intriguing possibility is that C and N transfers are linked together: although mycorrhizal fungi are the main N providers in all mycorrhizal plants, the very different ^{15}N abundance in mixo- and mycoheterotrophic plants, as compared to other autotrophic mycorrhizal plants (Figure 1b) [26], suggests that they receive N in a different form or via a different pathway. Ammonium, but also some amino acids, usually transfers N at the mycorrhizal interface to autotrophic mycorrhizal plants [1,27]. Could some organic molecules simultaneously provide N and C to mixotrophs? Indeed, N contents of mixotrophs are often high [2,4], but respiration alone can account for this by increasing the N:C ratio as compared to the food source [5]. The lack of correlation between ^{13}C and ^{15}N abundances among species (Figure 1a), as well as for a given species among sites [6], nevertheless suggests that some C is obtained through different, N-independent biochemical pathways.

Original mechanism(s) that might have evolved in mixotrophs to recover fungal C remain open to further investigation. The study of biochemical processes of C transfer has been hitherto limited by the unavailability of mixotrophic laboratory models due to their slow growth rates and the complicated biological systems they rely on (Figure 1b). Hopefully, some pyroloids can be outplanted [28]: although outplanting will destroy mycorrhizal links, it might allow the design of microcosms for investigating mixotrophy in controlled conditions to directly demonstrate C flow to mixotrophs and identify the underlying biochemical pathways.

Evolutionary predisposition to heterotrophy

Mixotrophy has important evolutionary implications. As previously mentioned, mixotrophic orchids and pyroloids are phylogenetically close to mycoheterotrophs that fully rely on fungal C (Box 1) [25,29]. Available phylogenies (e.g. Figure I in Box 1) show that mixotrophy appeared first and probably facilitated the emergence of mycoheterotrophy. The later transition probably arose through a shift to absolute dependence on fungal C. The ecological niche of mixotrophs might facilitate such transitions: mixotrophs often grow in young forests with loose canopy, where the C obtained from fungi compensates for the shade. Due to ongoing succession, the canopy of such stands tends to close as the forest develops, and shade increases. Mixotrophic populations therefore undergo continuous selective pressure for more light-independent C supply, which might lead to selection for mycoheterotrophy. A similar claim was made for mycoheterotrophs in the Burmanniaceae that evolved and diversified during the Eocene (between 56 and 34 myrs ago): at that time, high global temperatures enhanced expansion of tropical forests, and Burmanniaceae's autotrophic ancestors, growing in savannas, probably adapted to forest life through mycoheterotrophy [30].

Interestingly, in some mixotrophic orchids, non-chlorophyllous variants survive over years (Figure 2) [25,31]. Their ^{13}C abundance demonstrates that they are fully mycoheterotrophic [4,29]. They have no photosynthetic ability and respire less than green individuals (Figure 2) [4]. Their phenotype is stable over years, suggestive of either a genetic or an environmental determinism due to lasting local soil conditions [29]. Interestingly, such variants remain rare and produce fewer seeds because they dry before fruit ripening [4]. Causes for this reduced fitness remain to be investigated, but non-chlorophyllous variants are likely to represent unique snapshots of failed transitions from mixotrophy to mycoheterotrophy. They are ecological equivalents to mutants in genetics, that is, their dysfunctions might suggest what makes mycoheterotrophy successful. Although their determinism remains unknown,

Table 1. Becoming heterotrophic by exploitation of the living source that provides mineral nutrients: a convergent scenario for evolution of heterotrophy in plants through mixotrophic steps

Steps	Evolution to parasitism on other plants	Evolution to mycoheterotrophy	Positively selected for
# 1	Free-living, autotrophic mycorrhizal plant		
# 2	Hemiparasitic plant tapping xylem of its host mainly for mineral nutrition	Mineral nutrition by mycorrhizal fungi shared with surrounding plants that also contribute to fungal nutrition	Improved and less costly mineral nutrition
# 3	Hemiparasitic plant tapping xylem (mineral nutrition) and phloem (partial carbon nutrition) of its host, but still photosynthetic (parasitic mixotrophy)	Mixotrophic plant deriving mineral nutrients and some carbon from shared mycorrhizal fungi, but still photosynthetic (mycorrhizal mixotrophy; Figure 1a)	Improved carbon nutrition, improved tolerance to low light
# 4	Mixotrophic hemiparasitic plant with reduced photosynthetic abilities (smaller leaves, less pigments, etc.); better growing under its host	Mixotrophic mycorrhizal plant with reduced photosynthetic abilities (smaller leaves, less pigments, etc.); better growing in forests	Improved carbon nutrition in low light
# 5	Heterotrophic, non-green plant, obtaining all its mineral and carbon supply by tapping xylem and phloem of its host	Mycoheterotrophic, non-green plant obtaining all its mineral and carbon supply from its mycorrhizal fungi (Box 1)	Improved carbon nutrition, even in absence of light

they offer fascinating models for comparing the physiology of mixo- and mycoheterotrophs within similar genetic backgrounds.

Strikingly, the mixo- to mycoheterotrophy shift scenario is reminiscent of that leading to heterotrophic parasitic plants, which obtain photosynthates from host plants via a haustorium. In two lineages at least, the Orobanchaceae [32] and Convolvulaceae [33], phylogenies support the hypothesis that parasitic heterotrophs repeatedly arose from photosynthetic hemiparasitic ancestors. Interestingly, as previously mentioned, several hemiparasites are mixotrophic, receiving some organic C from their host [12]. This suggests a common scenario (Table 1), where a C flow emerges in the framework of biological interactions formerly selected by mineral needs. The resulting mixotrophy, in turn, allows emergence of heterotrophy, now selected by C needs only.

Undiscovered mixotrophs

Mixotrophy evolved convergently in two plant taxa (pyroloids and orchids), probably to enable survival in the forest understory. Can we expect discoveries of more mixotrophic taxa? Following the model in Table 1, mycoheterotrophy in a family is a strong predictor for mixotrophic species. Indeed, mycoheterotrophy arose at least 40 times in unrelated plant families [7] where, with detailed phylogenies at hand, one could search for mixotrophic species. Autotrophic and mycoheterotrophic species co-occur in many families that are excellent candidates for encompassing some mixotrophic species: Gentianaceae [34], Dioscoreales [35], Polygalaceae [36], Iridaceae [37], Pandanales [38] and Petrosaviaceae [39]. Of course, one can imagine that mixotrophic ancestors of some mycoheterotrophic lineages did not leave any extant mixotrophic descendants. At the same time, we might also discover mixotrophic clades that never shifted to mycoheterotrophy.

Another feature shared by mixotrophic pyroloids and orchids might also predict mixotrophy in other plant families. They produce a very large number of small, sub-millimetric seeds that are devoid of reserves. Germination of the undifferentiated embryo requires colonization by the future mycorrhizal fungus for its complete nutrition [1]. Until expansion of the first green leaves, subterranean seedlings are transiently mycoheterotrophic, probably using the same mechanism to recover

fungal C as mixotrophic adults. Although this syndrome might not characterize all mixotrophs, it is likely to indicate a predisposition to mixotrophy at adulthood. Among non-flowering plants, such as ferns and clubmosses, the small spores of several taxa develop into subterranean, heterotrophic gametophytes receiving C from their mycorrhizal fungi [40]. Recently, an association with mycorrhizal fungi that simultaneously colonize surrounding autotrophic plants was demonstrated for subterranean stages of the fern *Botrychium* [41] and the clubmosses *Lycopodium* and *Huperzia* [42]. Obviously, adult plants in these taxa are candidate mixotrophs and deserve further attention.

Finally, all investigations so far have focused on forests from temperate, Mediterranean and boreal regions, where mixotrophs are sometimes abundant (Figure 1a). Dense forests, strongly selecting for alternative C sources among understory species, cover large areas in the tropics and harbour a high biodiversity. Not surprisingly, the largest numbers of mycoheterotrophic species, although poorly studied so far, occur in tropical forests [7]. Mixotrophic species might also have diversified in tropical environments, and isotopic analyses are excellent tools for looking for them; in such analyses, a special focus should be placed on the families mentioned above.

The ecophysiological meaning of mixotrophy

Important questions remain open on the biology of C transfer. First, the cost for surrounding autotrophic plants and fungi is unknown. We do not know whether we face a parasitism or a somehow balanced exchange, which could occur, for example, if mixotrophs compensate by providing some vitamins or a 'shelter' (physical or chemical protection) for the fungus. Second, environmental factors influencing the level of use of fungal C by mixotrophic plants are also unclear. Many ecological or microcosm studies conducted on mixotrophic algae revealed that at least two factors drive the level of heterotrophy in mixotrophic planktonic species [43]: light deficiency favours phagocytosis as a source of C, whereas low nutrient levels favour phagocytosis as a source of N and P. The first surveys from orchid data suggested that light level was inversely correlated with dependence on fungal C [2,29], a trend that was also observed in labelling experiments reporting C transfers among plants linked by common mycorrhizal fungi [16,17]. But this intuitively expected correlation, where

fungal C compensates for lower photosynthesis, was not observed over a wide geographical range for pyroloids [6]. Just as for algae, plant mixotrophy might not always be based on C needs, but sometimes arises as a 'side-product' of the N and P nutrition. And this might explain the apparent discrepancies: according to the scenario in Table 1, organisms might (or might not) shift over evolutionary time from a situation where C is simply hitchhiking with organic N and P to a situation where C itself is the main nutrient recovered. At the first step, mineral availability determines the use of fungal C, whereas at the second step, C availability (and thus light) becomes the driving factor. We might expect that, for some mixotrophic species or families, soil mineral availability regulates dependence on fungal C. Similarly, carnivorous plants vary their production of trapping devices in response to N and P availability [44]. Comparative analysis of several plant families and sites will allow testing of this hypothesis in various phylogenetic backgrounds.

Mixotrophy based on mycorrhizal fungi is an exciting, newly discovered strategy that lies inbetween auto- and heterotrophy. A main goal will be to fully account for the C budgets of mixotrophic plants over the full growth season. Another pending question is the link with experimental approaches demonstrating short-term C transfers by C labelling between plants mutually sharing mycorrhizal fungi [16,19]: do these C transfers represent more than instantaneous transfers, perhaps reversible during the growing season [45], or do they represent a significant and net contribution to the C budget of receiving plants, which would therefore be mixotrophic?

Mixotrophy emphasizes the importance of mycorrhizal fungi and the sharing of common fungi with neighbours (Figure 1b) in plant physiology. Its discovery raises many questions. Is mixotrophy common in ecosystems on a global scale? Did it often evolve into mycoheterotrophy, and if so, how? What ecological factors determine the dependence on fungal C? What are the cellular mechanisms whereby C is transferred to receiving plants? Beyond *in situ* analyses of stable isotope contents and mycorrhizal fungal associates, easily tractable and relevant models, such as microcosm cultures, might enable broader experimental approaches in the near future.

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Plant Science Conferences in 2009

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